Enhancing Water Use Efficiency in Corn

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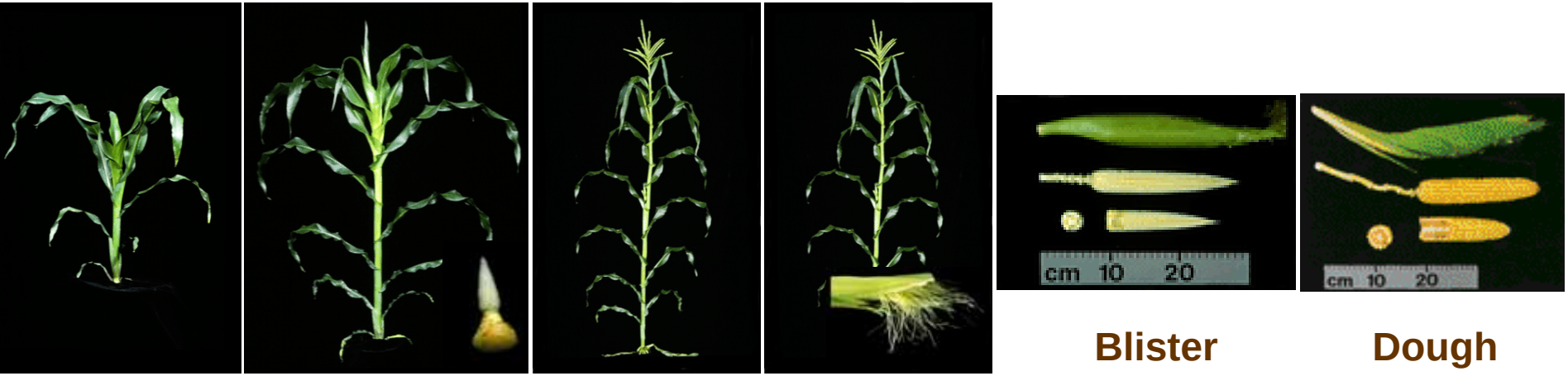
Impact of drought on maize yield



Blister Dough

Early vegetative Late vegetative Pollination Grain fill

5-10 % 10-25% 40-50% 30-40% 20-30%

Percent yield reduction associated with water deficits

(four consecutive days of visible leaf rolling)

From: Claassen, M.M., and R.H. Shaw (1970) *Agron. J.* 62:652-655

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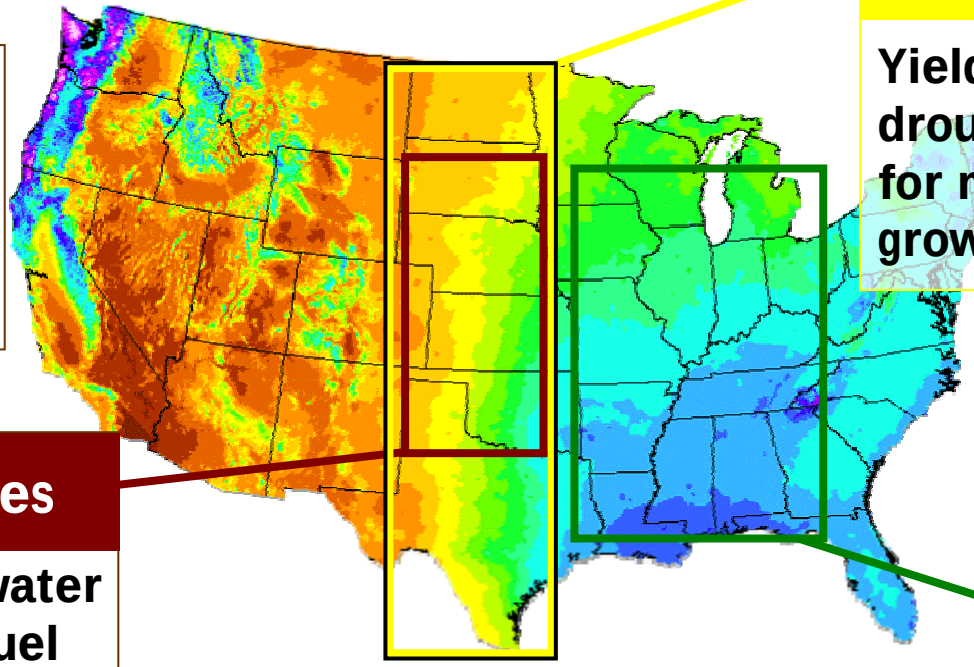
Farmer Needs for More Reliable and Efficient Production Define the Product Concepts and Drive Our Discovery Process

IRRIGATED

WESTERN DRYLAND

CENTRAL CORN BELT



Western Dryland Acres

Yield benefits due to drought trait / Opportunity for more crop choices for growers

Irrigated Acres

Protect aquifer water levels / Reduce fuel consumption needed to pump water

Low annual precipitation

High annual precipitation

Central Corn Belt

Yield Stability / Consistency

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Plants activate a diverse set of physiological, biochemical and molecular responses to drought leading to adaptation

Drought Stress

Physiological Responses

- Decreased Turger
- Reduced leaf WP (y)
- Decreased Stomatal Conductance
- Reduced Internal CO_2
- Decrease in net PS
- Osmotic Adjustment
- Reduced Mitosis
- Decreased LER

Biochemical Response

- ABA production & transport
- Decreased RUBISCO activity
- Decreased RuBP
- Decreased SPS, FBPase, PEPcase
- Production of ROS
- Accumulation of compatible solutes, amines, sugars, sugar alcohols
- Increase in Antioxidants
- Decrease in ROS

Molecular Responses

- Signal Perception
- ABA synthesis genes induced
- ABA responsive genes induced
- Upregulation of Stress-Protective genes (hsp/Chaperones,LEA, RAB, dehydrins ect)
- Upregulation of regulatory genes (TFs)

Drought Adaptation

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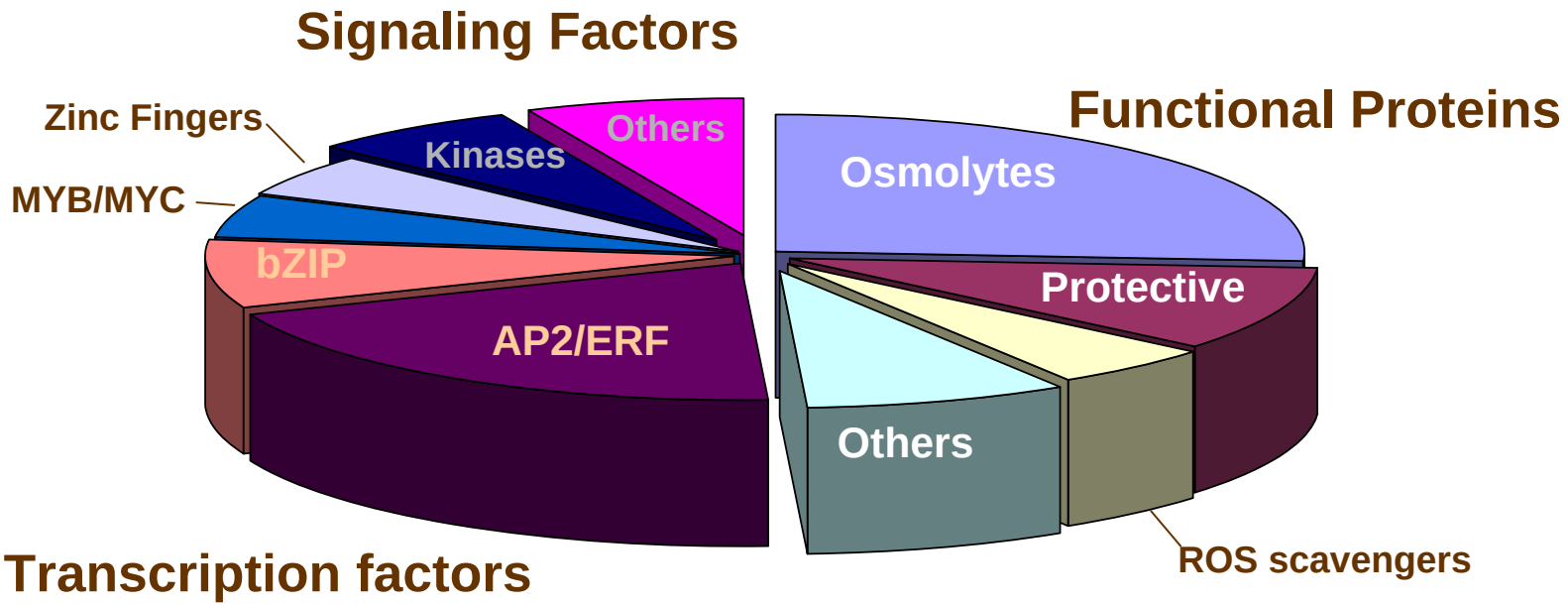
Diverse Gene Families Have Demonstrated Qualitative Drought Tolerance in Transgenic Plants: Models & Crops

Signaling & Transcriptional Control

Membrane protection, osmoprotectants & antioxidants



From: Umezawa et al 2006 Current Opinion in Biotechnology 17:113

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Drought Tolerant Crops

- Conventional Breeding
- MAS/QTLs
- Plant Biotechnology

→

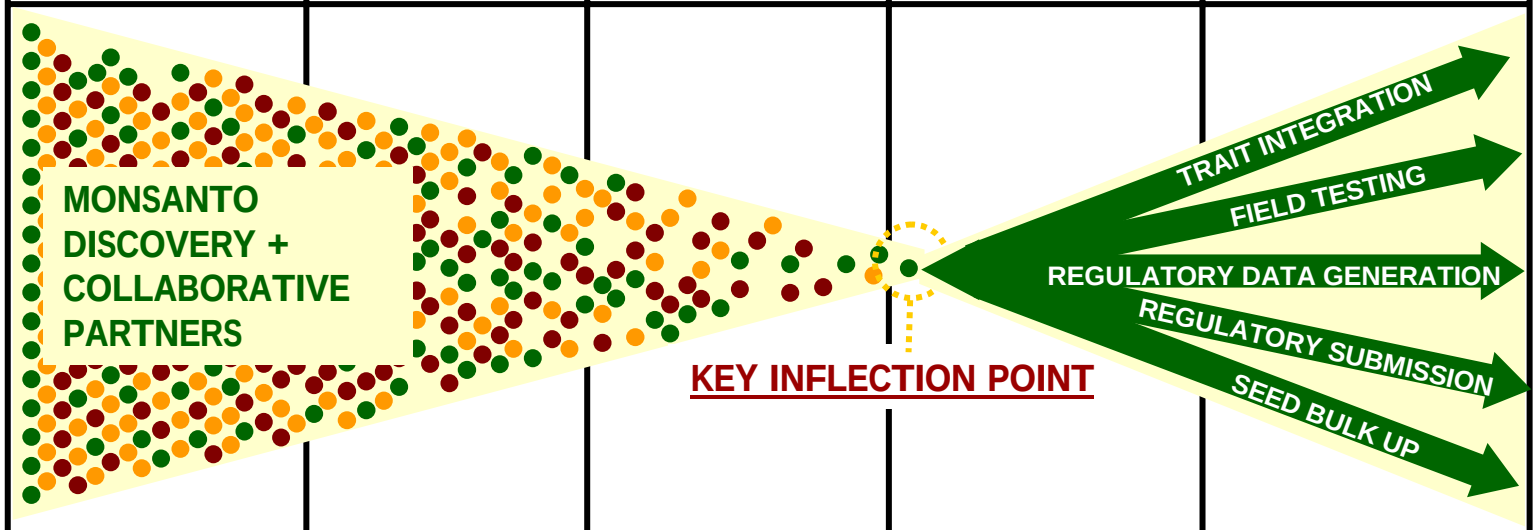
* Stress Tolerance Proteins
* Response regulators
* Stress associated metabolites

Lead to:

- Step changes in yield with transgenic trait
- Impact multiple genes / pathways
- Breeding for secondary traits/target market adaptation

Phases of Product Development: identifying product candidates from discovery efforts

	DISCOVERY **Gene/Trait Identification**	PHASE I **Proof Of Concept**	PHASE II **Early Development**	PHASE III **Advanced Development**	PHASE IV **Pre-launch**
AVERAGE DURATION[1]	24 to 48 MONTHS	12 to 24 MONTHS	12 to 24 MONTHS	12 to 24 MONTHS	12 to 36 MONTHS
GENES IN TESTING	TENS OF THOUSANDS	THOUSANDS	10s	<5	1



1. Time estimates are based on our experience; they can overlap. Total development time for any particular product may be shorter or longer than the time estimated here.

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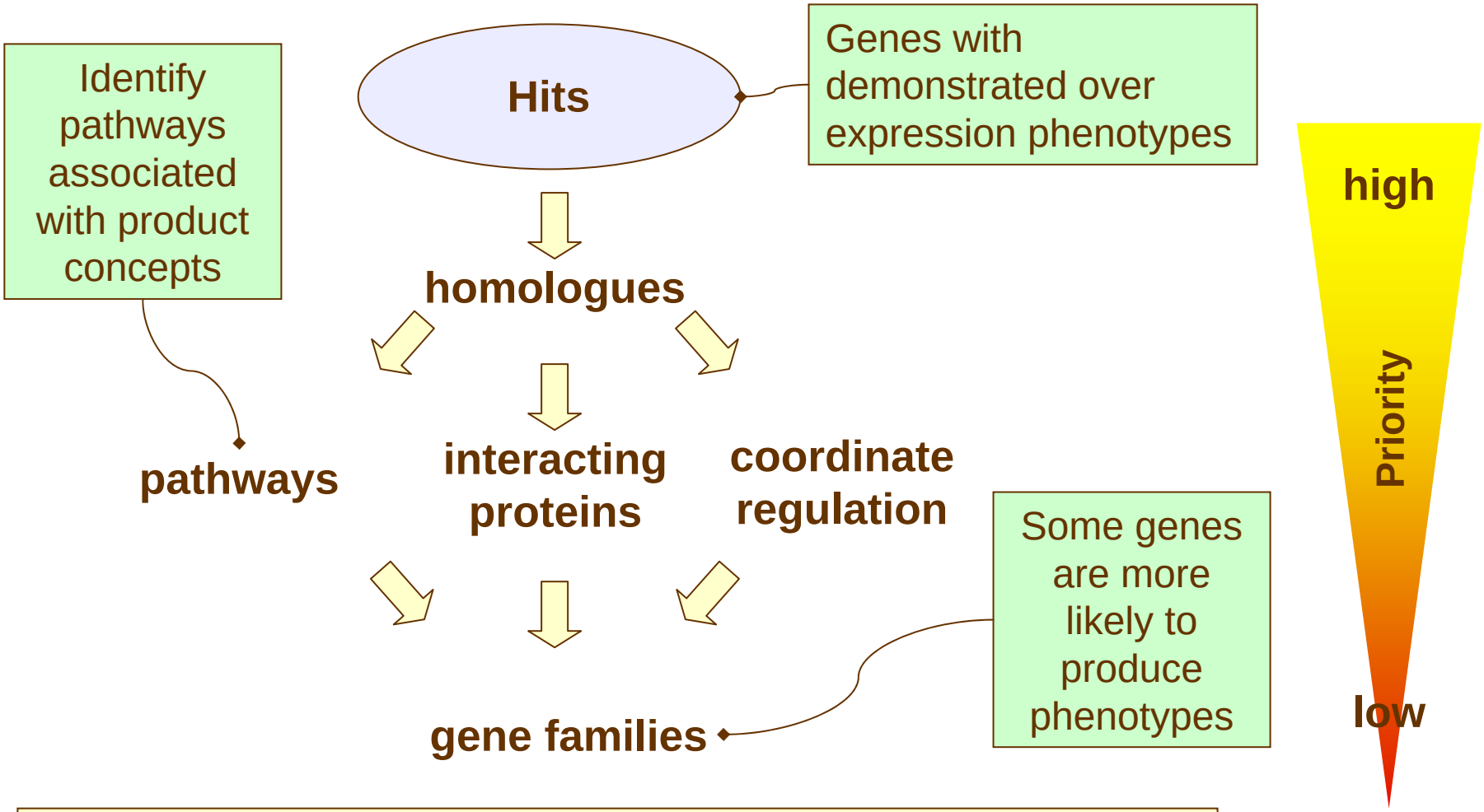
Gene selection is primarily driven by model system results

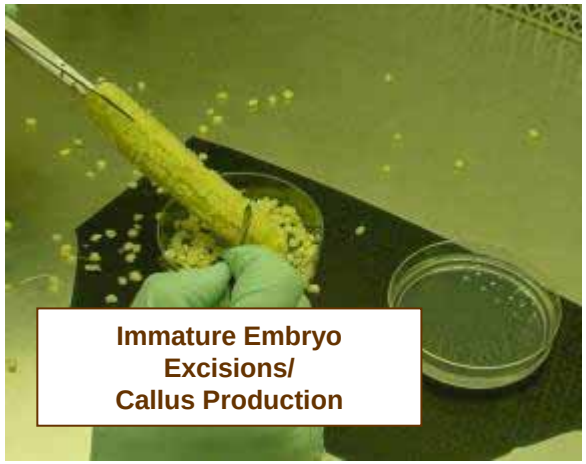


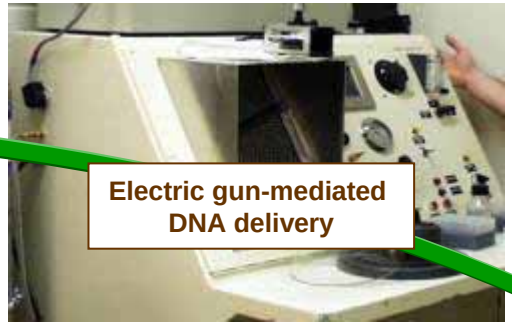
Depth of sampling depends on depth of pipeline, strength of evidence and expected value of product

Additional Criteria for Gene Selection

- Systematic search to avoid genes with similarity to known allergens or toxins
- IP status reviewed
- Duplicates filtered out

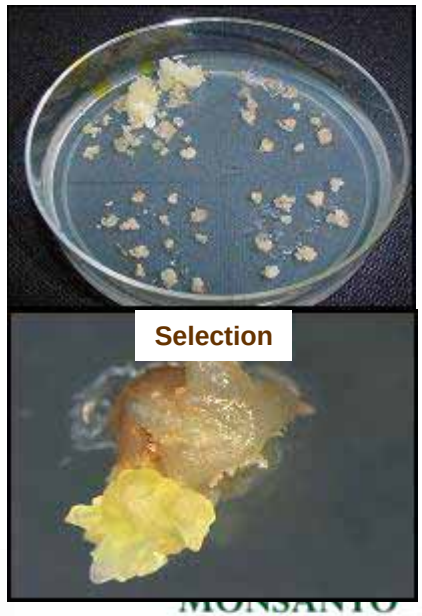
Sequence identified → Allergen screen → Toxin screen → IP screen → Duplicate screen → Cloning





High Throughput Corn Transformation

Thousands of events produced annually



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HTP Technologies Support Molecular Analysis

-mRNA Expression Levels

-QC of plants

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Multi-season Breeding Process to produce seed for screens and testing

R0
↓ RP
BC1F1 — Seed → **Archive**
↓ Self
BC1S1 — Seed ⇢ **Early Inbred Evaluation**
- Seed analytics
- Trait teams

↓ Self
BC1S2 (+/+) — Seed → **Inbred/Hybrid Evaluation**
- approved sites
- Seed analytics
- Trait teams

↓ Tester
Hybrid — Seed → (Trait teams)

Identifying Lead Genes for Drought Tolerance Using Functional Genomics Platform

- High Throughput Cloning, Transformation, Greenhouse & Field Screens
  - Detailed Physiology Trials
    - Controlled Drought Field Yield Trials
      - Multiple Location & Germplasm Yield Trials

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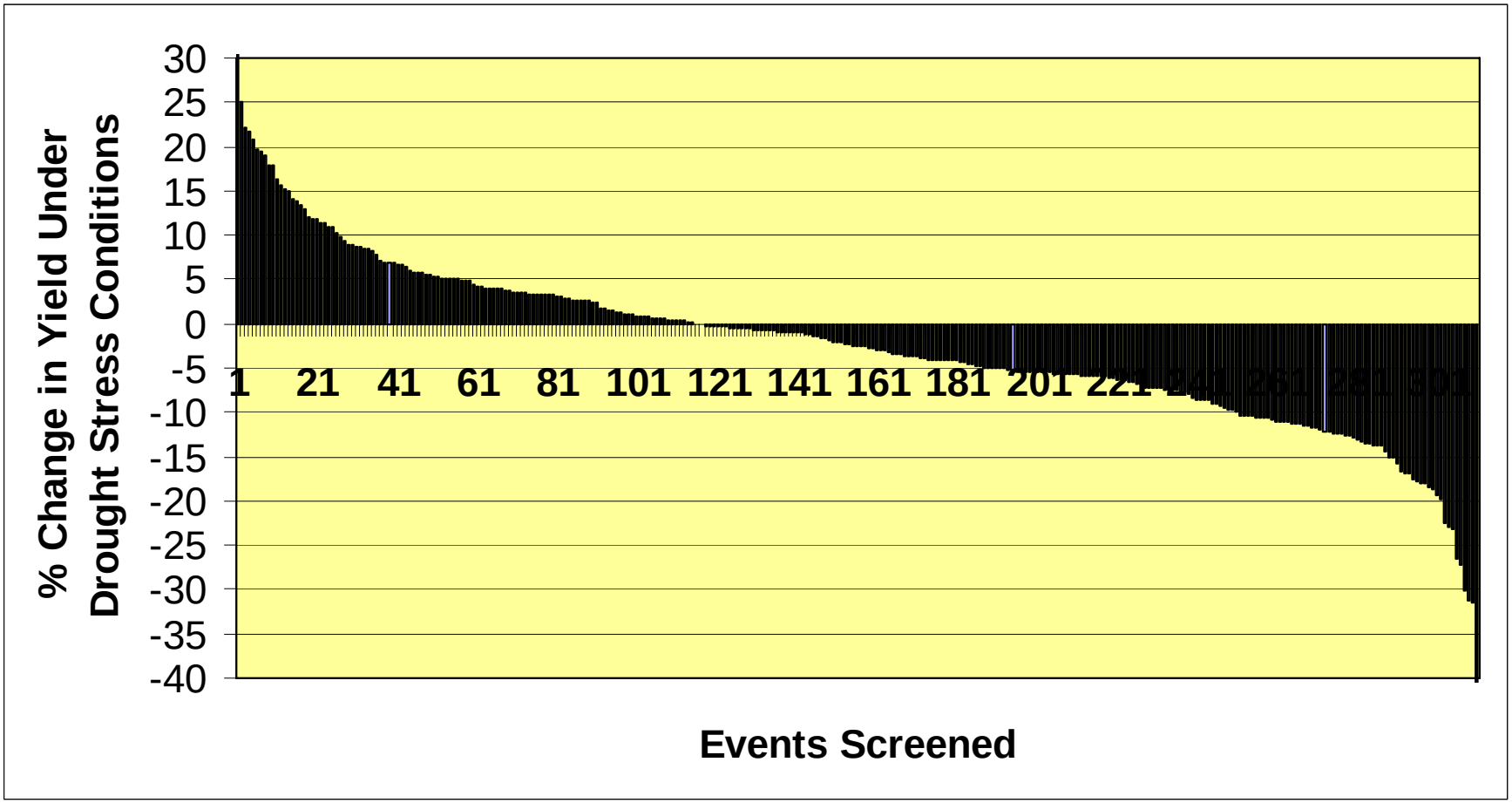
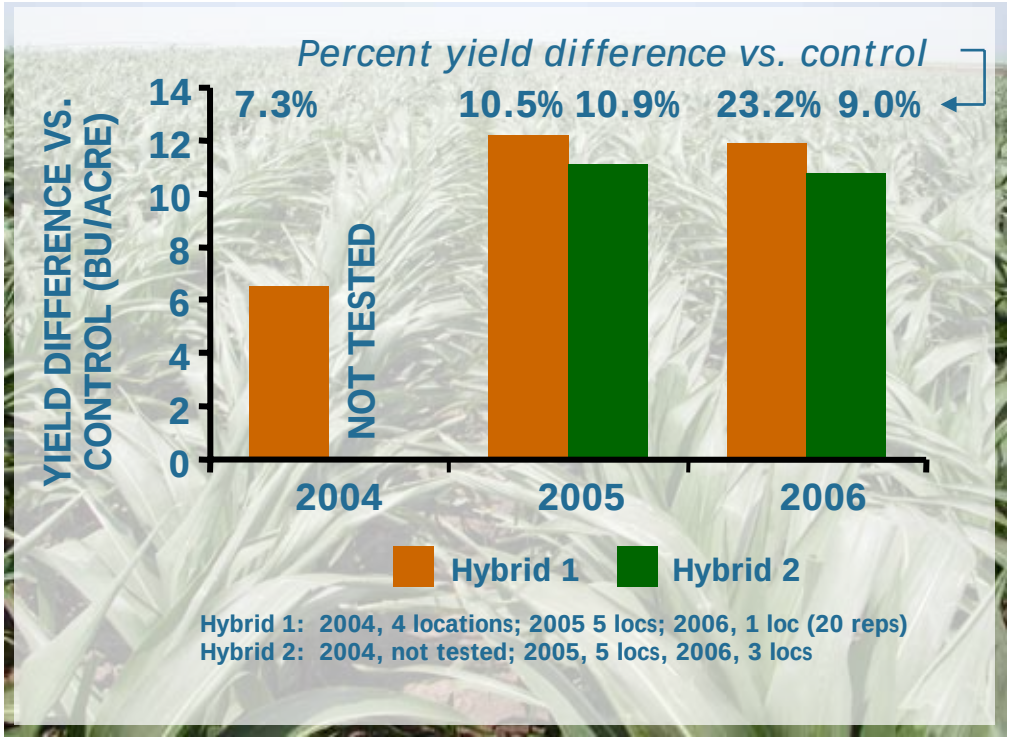
Transgenes create variation with respect to yield under drought:

Yield under Controlled Drought Key Measure for Advancement



Genes represented in this trial had demonstrated GH drought efficacy

Yield and Productivity Remain the Keys to Successful Drought Tolerance Trait



- Gene **+ Gene**

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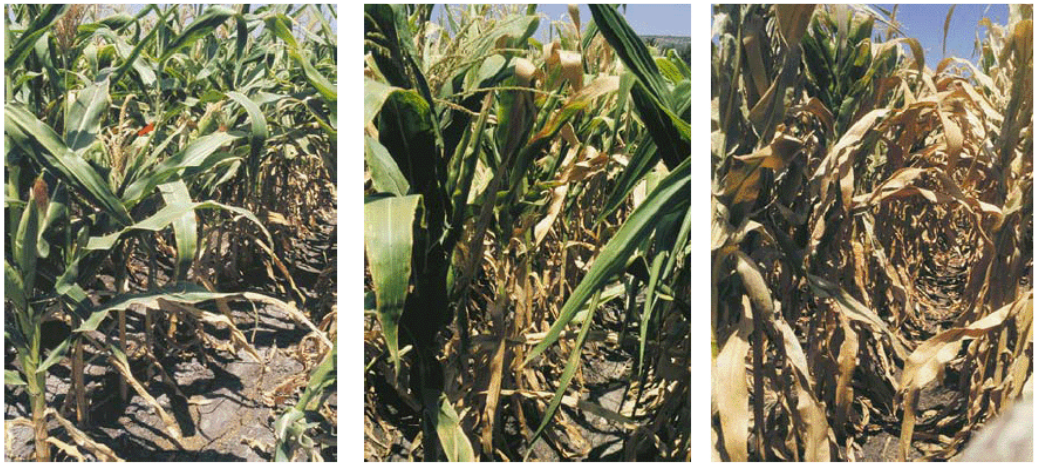
Focus on Traits Associated with Yield Under Drought

Chlorophyll content

Leaf rolling

ASI = SD - AD



Senescence, stay-green

Tassel size

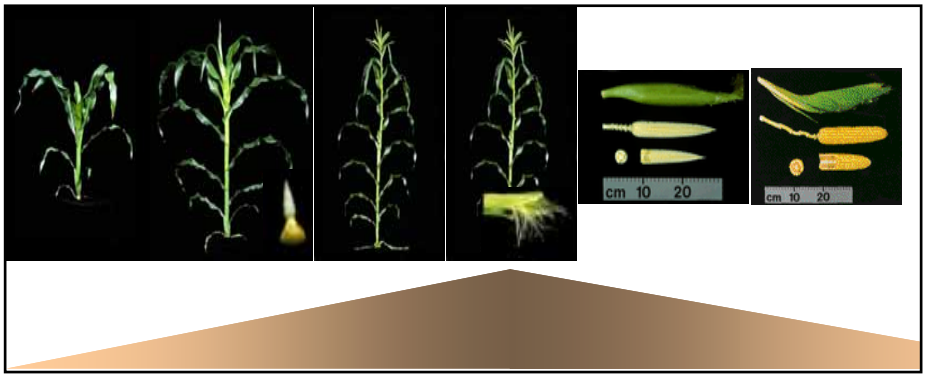
Leaf firing, tassel blasting

Growth

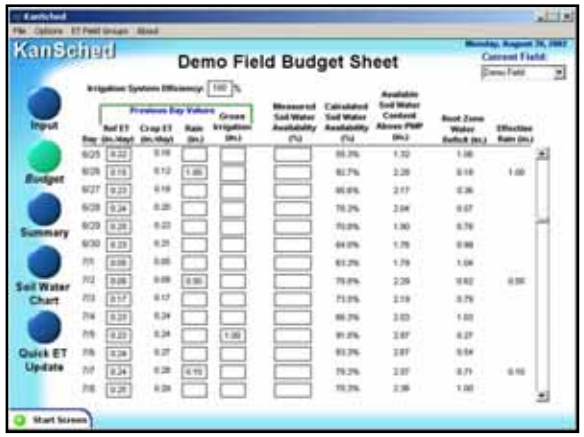
Betran, 2005

Additional Traits: Grain Set, Grain Fill, Kernel Abortion, etc.

Consistent replication of drought stress is the key to finding new genes

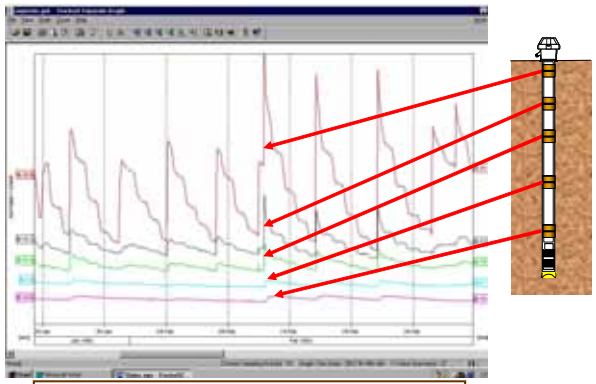


Stage of Development.



Water Supply Budgeting Tools

Rainfall &
Evapo-trans. Data.



Soil Moisture
Measurements

Plant Stress Observations

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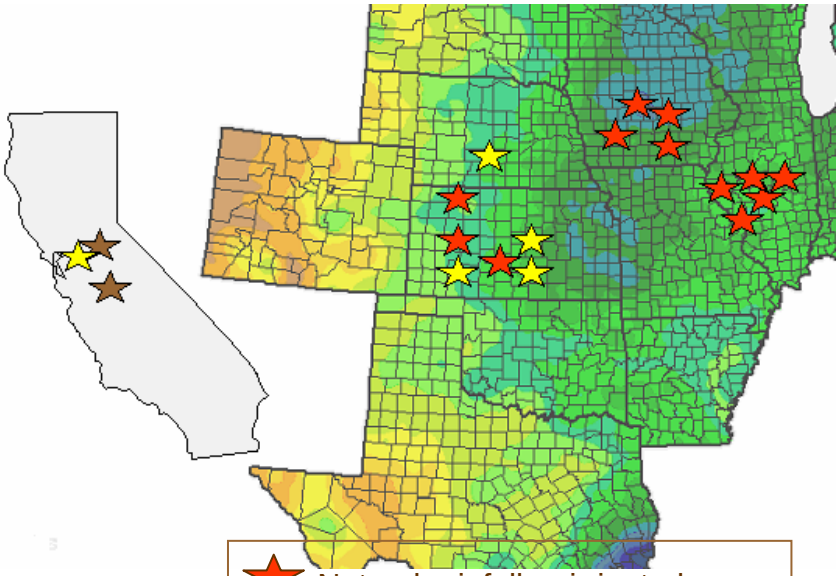
Reiterative Field Testing under Target Water Stress, Multiple Environmental Data Points To Identify Lead Lines for Advancement

Corn Drought Yield Testing Network

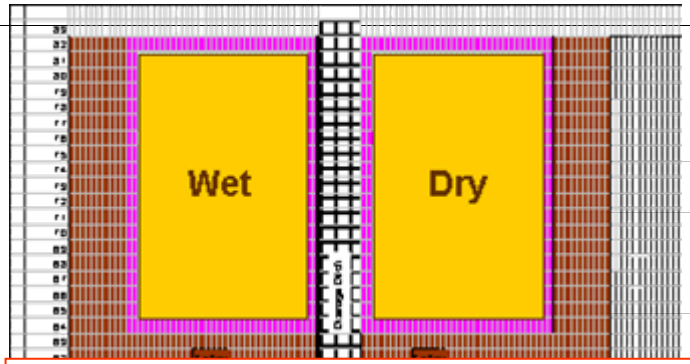


Natural rainfall or irrigated

Controlled drought stress

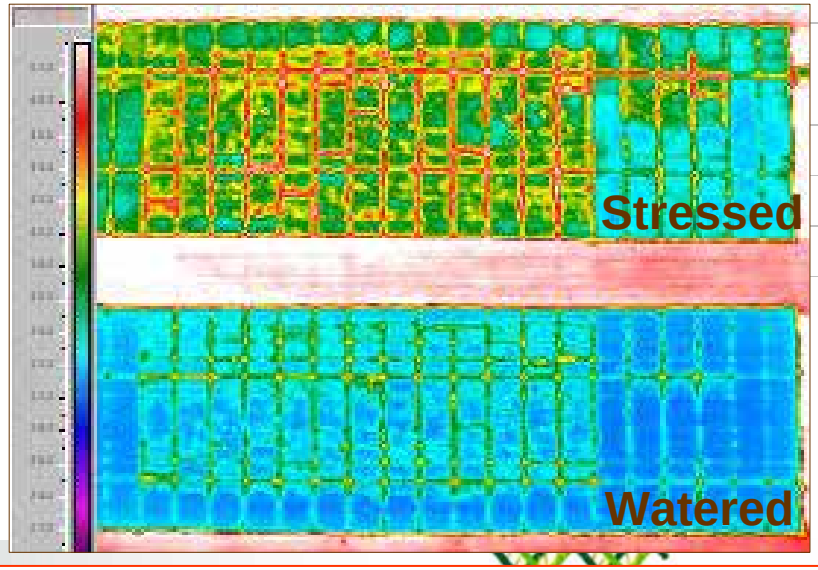
Controlled drought & full irrigation

Multiple Locations



Multiple Replications per Location

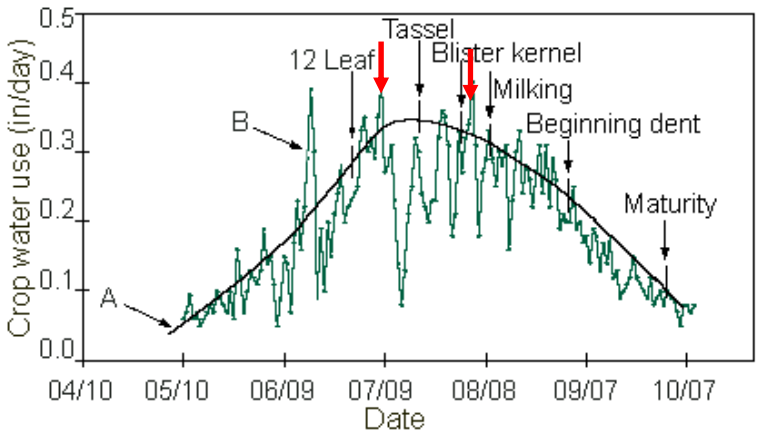
Aerial photo of leaf temperature



Multiple Plant & Environment Measurements

Associated traits important for drought tolerance

- Ideal Secondary Traits need to be highly correlated to yield under stress, carry no yield penalty under favorable conditions, be highly heritable, cheap and easy to measure, stable over time, and be observed at or prior to flowering
- Corn grain yield is most sensitive to water and heat stress from just before silking through grain fill



Key secondary focus traits:

- Protogyny or a negative anthesis-silking interval (**ASI**)
- Increased prolificacy (reduced barrenness)
- Improved staygreen
- Reduced tassel size
- Larger ears prior to anthesis
- Rooting depth and structure
- Shorter plants at flowering
- Upright leaf habit

Disease concerns:

- Resistance to *Aspergillus flavus* infection and aflotoxin contamination – high temperatures and drought have been reported to increase growth of the fungus and toxin production (Payne, 1998)
- Resistance to Goss's Wilt

Product development testing: Research conducted to advance discovery leads to commercial traits

- Multiple-Year Field Trials:
  - Progression in numbers of environments
    - Regulated by USDA/APHIS
  - Germplasm combining ability

- Evaluate stacks with Existing Commercial Traits and Germplasm

- Conduct Regulatory Science studies required to support applications to appropriate agencies.

- Integrate traits into elite germplasm and existing commercial stacks at time of launch.

Drought Tolerant Corn – Summary

- A discovery and development pipeline established:
  - **A pipeline for traits that improves yield by improving water utilization efficiency**
- Making progress:
  - Multiple genes **provide degrees of yield stability / drought stress tolerance.**
- Now - intensive testing :
  - **more environments, assessing stacked with existing traits in best adapted germplasm - test fit to each market segment; and commercial event selection.**

Thank You

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